REMARKS

Applicants have amended claim 1 to make explicit that which was implicit. Namely, first that "the reactive species" in step (2) refers to either "the reactive oxygen or the reactive nitrogen species" that were specifically referred to in step (1). As such, the amendment is clerical. Second, Applicants have amended claim 1, step (3) to refer to the specific functions that the hepatocytes will retain, which are specifically listed at in paragraph [0039] of the specification. Applicants have also amended claims 5 and 7 to specify that the glutathione precursor is 2-oxo-thiazolidine-4carboxylate, as discussed, e.g., in paragraph [0046] of the specification. Claims 6 and 8 have been amended to spell out the well known chemical formula for N^G-methylarginine as indicated, *supra*. Accordingly, no new matter has been introduced by the virtue of these amendments and their entry is respectfully requested.

The Examiner rejected claims 1-8 under 35 USC 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter. Specifically, the Examiner noted that the term "the reactive species" in claim 1 lacks sufficient antecedent basis.

Applicants have amended claims as described, *supra*. In view of the amendments, Applicants submit that the rejection has been obviated and should be withdrawn.

The Examiner also alleged that the chemical name for the claimed glutathione precursor compound such as "2-oxo-thiazolidine" is incorrect and/or indefinite.

Applicants have amended claims as described, *supra*. In view of the amendments, Applicants submit that the rejection has been obviated and should be withdrawn.

The Examiner further alleged that the chemical name of the presently claimed "N^G-methylarginine" is also incorrect and/or indefinite.

Applicants respectfully disagree. N^G-methylarginine (N^G-MeArg) is a well known compound, wherein the superscript "G" refers to the guanidine moiety (see, e.g. Exhibit A attached hereto). A skilled chemist can easily draw the chemical formula for a methylarginine

that has a guanidine moiety attached thereto. However, to expedite prosecution, Applicants have amended the claim to make the formula explicit.

Accordingly, in view the amendments, Applicants submit that the rejection has been obviated and should be withdrawn.

Therefore, Applicants submit that the rejection of claims 1-8 under 35 USC 112, second paragraph, should be withdrawn.

Claims 1, 2 and 4 are rejected under 35 USC 102(b) as being anticipated by US 5,198,432 (Fariss) in light of evidence by ATCC catalogue and Sharma et al.

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

Fariss discusses that alphatocopherol added to Waymouth's medium protects primary hepatocytes when the cells are exposed to a toxic chemical (EMS, 50 mM; or ionphore A-23187, 5 μM). The Examiner relies on the ATCC catalog as showing the ingredients of Waymouth's medium which contains folic acid. The Examiner further relies on Sharma to show that folic acid was allegedly a known to increase intracellular glutathione levels.

However, the claims require that the hepatocytes are grown in the presence of the claimed supplements and actually **maintain their function** for **at least 5 days**. The specification further teaches that maintaining functional hepatocytes for longer periods of time has been a significant problem (see, e.g. paragraph [008] of the Specification). Fariss does not teach a method of culturing primary **functional** hepatocytes for **at least 5 days.** Applicants have also amended the claim to explicitly state what functions must be preserved. Nowhere does Fariss teachs a method of culturing the hepatocytes in the presence of these 2 compounds for at least five days, and **maintaining their ability to metabolize xenobiotics or cytochrome P450 gene expression, or that display fenestration, bile canniculi or binucleation** as taught in the present application (see, e.g. par. [0039]). Figures 1 and 2 of Fariss indicate that they observed the cells in culture only for **5 hours**—not five days. Nothing in the secondary references suggest culturing the hepatocytes for at least 5 days.

Therefore, Applicants respectfully submit that the rejection of claims 1, 2 and 4 under 35 USC 102(b) over US 5,198,432 (Fariss) in light of evidence by ATCC catalogue and Sharma et al. should be withdrawn.

The Examiner rejected claims 1-8 under 35 U.S.C. §103(a) as allegedly obvious over Fariss as evidenced by the ATCC catalogue and Sharma et al., and in view of Pourahmad et al., Dilworth et al., and Roberts et al..

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As discussed, *supra*, Fariss does not teach a method for culturing primary hepatocytes for at least 5 days and maintain function.

None of the secondary references overcome this deficiency because none of them describe culturing the primary hepatocytes that retain their function for at least 5 days.

Pouhramad et al., describe effects of an environmental toxin, $CdCl_2$ on the life of hepatocytes. The maximum time they observed was **120 minutes**. They further state that the primary hepatocytes were incubated 30 minutes prior to the addition of the toxin. Thus the total life time for the hepatocyte cultures would have been a maximum of 150 minutes, or **2 hours and 30 minutes**.

Dilworth et al., describes that **increased level of nitric oxide (NO)** in hepatocyte cultures have an **inhibitory effect** on compound-induced apoptosis (last sentence of the abstract). Therefore, Dilworth concludes that one may have to take this effect into account when using primary hepatocytes in toxicological studies (page 629, col. 2, last par.). They also teach that further research is required to determine the full extent of the effects of NO in hepatocyte cultures. The maximum time Dilworth observed their cells was **72 hours**. Accordingly, nothing in Dilworth teaches or suggests that NO inhibitors could let alone should be added to extend the life of primary hepatocytes.

Roberts et al. describe that they exposed the hepatocytes to the compounds only for **4 hours** (see, page 1893, col. 2, under Biological Results). Moreover, the cells in Roberts' experiments were in a suspension, not in a monolayer culture (see, articles cited in Roberts:

Seglen P.O. Exp. Cell Res., 1972, 74:450; Seglen P.O. Exp. Cell Res., 1973, 75:25; and Seglen P.O. Exp. Cell Res., 1973, 82:391, Exhibits B-D attached herewith)

The Examiner further argued that one would have

> "a reasonable expectation of success in maintaining **viability of hepatocytes** because the presently claimed agents have been known and used for **culturing and maintaining viability** of hepatocytes as adequately demonstrated by the cited references combined." (Emphasis added)

However, this is not what is claimed. The claims are not directed to a mere maintenance of viability. What is claimed is a method wherein one cultures the hepatocytes for at least 5 days and not only maintains viability but **also maintains specific functions** of primary hepatocytes **for at least 5 days**. The present invention is based on the surprising finding that the combination of the specific agents as claimed results in conditions, wherein primary hepatocytes actually maintain their function for long periods of time (see, e.g., par [0038]).

Therefore, in view of the above and the amendments to the claims, Applicants respectfully submit that the rejection of claims 1-8 under 35 USC 103(a) over Fariss in light of evidence by ATCC catalogue and Sharma et al. taken with Pourahmad et al., Dilworth et al., and Roberts et al. should be withdrawn. These references actually suggest that you would not use these compounds to culture hepatocytes for at least 5 days.

The Commissioner is herewith authorized to charge fee deficiencies and credit overpayments to the NIXON PEABODY LLP Deposit Account No. 50-0850.

In view of the foregoing, Applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is requested.

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Respectfully submitted,

Customer No. 40679

/Leena H. Karttunen/

Ronald I. Eisenstein (Reg. No. 30,628)
Leena H. Karttunen (Reg. No. 60,335)
NIXON PEABODY LLP
100 Summer Street
Boston, MA 02110
(617) 345-6054 / 1367